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15/63, 15/67, 15/85, C07K 14/54, A61K 31/713

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DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
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LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ,
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IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

— Before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND COMPOSITIONS FOR PREVENTING THE FORMATION OF ABERRANT RNA DURING
TRANSCRIPTION OF A PLASMID SEQUENCE

(57) Abstract: Polynucleotide molecules, which include single stranded DNA or RNA, partially double-stranded DNA, and double-stranded DNA molecules, contain terminator sequences and/or other modifications which suppress the production of unwanted polynucleotide species from these molecules when transfected in a host cell. These molecules are useful in methods for enhancing the efficiency of transcription of a selected polynucleotide sequence in a transfected host cell, and reducing the potential for the products of unwanted transcripts. Further, the methods of the invention are useful in avoiding extinguishing or down regulating the expression of certain polynucleotides present in a host cell or host. These compositions and methods are useful in therapeutic, vaccine, diagnostic and research fields.

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INTERNATIONAL SEARCH REPORT

Internat. Application No.
US 00/17670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/24 C12N15/63 C12N15/67 C12N15/85 C07K14/54
A61K31/713

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, STRAND, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category * Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.

X	<p>ANDRE S ET AL: "INCREASED IMMUNE RESPONSE ELICITED BY DNA VACCINATION WITH A SYNTHETIC GP120 SEQUENCE WITH OPTIMIZED CODON USAGE" JOURNAL OF VIROLOGY, US, THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 72, no. 2, 1 February 1998 (1998-02-01), pages 1497-1503, XP002073767 ISSN: 0022-538X the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	31-35
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of the actual completion of the international search

25 October 2000

Date of mailing of the international search report

08/11/2000

Name and mailing address of the ISA
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Authorized officer

Hornig, H

INTERNATIONAL SEARCH REPORT

Internat. Application No.
US 00/17670

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZOLOTUKHIN S ET AL: "A HUMANIZED GREEN FLUORESCENT PROTEIN CDNA ADAPTED FOR HIGH-LEVEL EXPRESSION IN MAMMALIAN CELLS" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 70, no. 7, 1 July 1996 (1996-07-01), pages 4646-4654, XP002030427 ISSN: 0022-538X the whole document	31-35
X	WO 99 14346 A (SEQUITUR INC) 25 March 1999 (1999-03-25) the whole document	36-47
A	WO 99 10509 A (LIPMAN DAVID J ;GOVERNMENT OF THE UNITED STATE (US)) 4 March 1999 (1999-03-04) the whole document	
A	WO 97 44450 A (PEYMAN JOHN A ;UNIV YALE (US)) 27 November 1997 (1997-11-27) the whole document	
A	WO 95 29244 A (WISCONSIN ALUMNI RES FOUND) 2 November 1995 (1995-11-02) the whole document	
A	WO 96 19573 A (CANGENE CORP ;DELCUVE GENEVIEVE (CA)) 27 June 1996 (1996-06-27) the whole document	
A	ENRIQUEZ-HARRIS P ET AL: "A PAUSE SITE FOR RNA POLYMERASE II IS ASSOCIATED WITH TERMINATION OF TRANSCRIPTION" EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 10, no. 7, 1991, pages 1833-1842, XP002151088 ISSN: 0261-4189 cited in the application the whole document	
A	BAHRAMIAN MOHAMMAD B ET AL: "Transcriptional and posttranscriptional silencing of rodent alpha1(I) collagen by a homologous transcriptionally self-silenced transgene." MOLECULAR AND CELLULAR BIOLOGY, vol. 19, no. 1, January 1999 (1999-01), pages 274-283, XP002151089 ISSN: 0270-7306 cited in the application the whole document	

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INTERNATIONAL SEARCH REPORT

Intern: at Application No

T/US 00/17670

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAMERON F H ET AL: "INHIBITION OF GENE EXPRESSION BY A SHORT SENSE FRAGMENT" NUCLEIC ACIDS RESEARCH, vol. 19, no. 3, 1991, pages 469-475, XP002151090 ISSN: 0305-1048 the whole document ----	
A	L. TIMMONS AND A. FIRE: "Specific interference by ingested dsRNA" NATURE, vol. 395, 29 October 1998 (1998-10-29), page 854 XP002151091 MACMILLAN JOURNALS LTD., LONDON, UK cited in the application the whole document ----	
A	MISQUITTA LEONIE ET AL: "Targeted disruption of gene function in Drosophila by RNA interference (RNA-i): A role for nautilus in embryonic somatic muscle formation." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 96, no. 4, 16 February 1999 (1999-02-16), pages 1451-1456, XP002151092 Feb. 16, 1999 ISSN: 0027-8424 cited in the application the whole document ----	
A	NILSSON M ET AL: "PADLOCK PROBES: CIRCULARIZING OLIGONUCLEOTIDES FOR LOCALIZED DNA DETECTION" SCIENCE, US, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 265, no. 5181, 30 September 1994 (1994-09-30), pages 2085-2088, XP000579803 ISSN: 0036-8075 cited in the application the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interne Application No

T/US 00/17670

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9914346	A	25-03-1999	AU 9319398 A EP 1021549 A	05-04-1999 26-07-2000
WO 9910509	A	04-03-1999	AU 8916098 A	16-03-1999
WO 9744450	A	27-11-1997	US 6022863 A AU 3225897 A	08-02-2000 09-12-1997
WO 9529244	A	02-11-1995	US 5587300 A AU 2295995 A	24-12-1996 16-11-1995
WO 9619573	A	27-06-1996	US 5888774 A AU 692968 B AU 4169296 A CA 2208013 A EP 0800577 A US 5985607 A	30-03-1999 18-06-1998 10-07-1996 27-06-1996 15-10-1997 16-11-1999

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 29 OCT 2001

WiPO

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Applicant's or agent's file reference AHP29APCT		FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)
International application No. PCT/US00/17670		International filing date (day/month/year) 27/06/2000	Priority date (day/month/year) 09/07/1999	
International Patent Classification (IPC) or national classification and IPC C12N15/24				
Applicant AMERICAN HOME PRODUCTS CORPORATION				
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>				
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input checked="" type="checkbox"/> Certain observations on the international application</p>				
Date of submission of the demand 02/02/2001		Date of completion of this report 25.10.2001		
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized officer Burkhardt, P Telephone No. +49 89 2399 7456		



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/17670

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-44 as originally filed

Claims, No.:

1-54 as originally filed

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: -, which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US00/17670

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 30, 48 - 51
	No:	Claims	31 - 47, 52 - 54

Inventive step (IS)	Yes:	Claims	
	No:	Claims	1 - 51

Industrial applicability (IA)	Yes:	Claims	1 - 51
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/17670

R Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this report; the numbering is following the order of the International Search Report:

- D1 André *et al.*, 1998. J Virol. 72:1497-1503.
- D2 Zolotukhin *et al.*, 1996. J Virol. 70:4646-4654.
- D3 WO-A-9914346 (Sequitur Inc.)

1. Article 33(2) PCT (Novelty)

1.1 Present claim 1 is directed to a double stranded polynucleotide wherein the first and second strand comprise terminator sequences that are not present on the corresponding complementary strand. Both terminator sequences are, however, supposed to terminate transcription on the respective strand.

1.2 Such a polynucleotide has not yet been disclosed in the prior art presently available to the IPEA. Present claim 1 meets the requirements of Article 33(2) PCT. The same holds true for dependent claims 2 - 30 and for claims 48 - 51 directed to methods of employing said polynucleotides.

1.3 Present claim 31 is directed to a codon optimised double-stranded polynucleotide wherein the nucleotide sequence is non-homologous to sequences in the host cell.

Documents D1 (page 1497, "Abstract" and "Material And Methods") and D2 (page 4646, "Abstract" and "Material And Methods") disclose codon optimised DNA sequences that are non-homologous to sequences in the host cells. D1 and D2 thus anticipate the subject-matter of present claim 31. The same holds true for dependent claims 32 - 35 and for claim 46 directed to the corresponding RNA molecules.

1.4 Present claim 36 is directed to a single stranded RNA molecule that is

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/17670

incapable of forming partially double-stranded complexes.

Document D3 (Examples) discloses RNA molecules that have been modified to improve RNA stability which also comprises avoiding the formation of double-stranded complexes. D3 therefore anticipates the subject-matter of present claim 36. The same holds true for dependent claims 37 - 45 and 47 and for claims 52 - 54 directed to methods employing said single stranded RNA molecules.

2. Article 33(3) PCT (Inventive step)

2.1 Present claim 1 does not meet the requirements of Article 33(3) PCT. It is not apparent from the description that the subject-matter of claim 1 would solve the technical problem, namely to provide polynucleotide molecules that inhibit the formation of aberrant RNA and thus lead to an increased expression level. The examples found in the description do not provide such evidence. (Please also see the comments in section VIII below). The same holds true for dependent claims 2 - 30 and for claims 48 - 51.

2.2 The same objection applies to claims 41 - 47. Said claims do also not meet the requirements of Article 33(3) PCT for the reasons outlined above.

Re Item VIII

Certain observations on the international application

1. It appears as if the claims 1 - 30 relate more to a theoretical model than to subject-matter defined in terms of technical features. Such a set of claim does not meet the requirements of Article 6 PCT in conjunction with Rule 6.3(a) PCT.
2. Present claims 36 and 46 are unclear (Article 6 PCT). It is not apparent what the term "**substantially** single stranded RNA molecule" may comprise. Is it 90% single stranded or only 50% or is it meant to be interpreted in a completely different way?

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 05 April 2001 (05.04.01)	
International application No. PCT/US00/17670	Applicant's or agent's file reference AHP29APCT
International filing date (day/month/year) 27 June 2000 (27.06.00)	Priority date (day/month/year) 09 July 1999 (09.07.99)
Applicant SATISHCHANDRAN, C. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
02 February 2001 (02.02.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Antonia Muller Telephone No.: (41-22) 338.83.38
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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference AHP29APCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/17670	International filing date (day/month/year) 27/06/2000	(Earliest) Priority Date (day/month/year) 09/07/1999
Applicant AMERICAN HOME PRODUCTS CORPORATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

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☐ None of the figures.